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DIAPAUSE IN THE GEMMULES OF THE MARINE SPONGE, *HALICLONA LOOSANOFFI*, WITH A NOTE ON THE GEMMULES OF *HALICLONA OCULATA*[1]

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Haliclona loosanoffi is one of several marine sponges that produce special structures called gemmules (Hartman, 1958; Wells, Wells and Gray, 1964; Fell, 1974; and Simpson and Fell, 1974). Each gemmule consists of a mass of large granular cells enclosed within a collagenous capsule which may be fortified with spicules. In some cases such gemmules persist during certain unfavorable conditions and germinate, forming new sponges, when more favorable conditions recur. For example, in Southern New England, specimens of *Haliclona loosanoffi* degenerate during the late summer and early fall, leaving exposed on the substrate gemmules that were produced at the bases of the sponges. During the winter when the water temperature may fall to below 0° C and small protected bodies of water may be covered by ice for several weeks, this sponge occurs only in the form of gemmules. These gemmules germinate in late spring when the water temperature rises to about 20° C (Hartman, 1958; and Fell, 1974). On the other hand, at Hatteras Harbor, North Carolina *Haliclona loosanoffi* is abundant during the winter when the water temperature drops to at least 5° C. There this sponge is found exclusively in the form of gemmules during part of the warm summer (Wells *et al.*, 1964).

In view of the apparent relationship of gemmule germination to water temperature, two types of studies were undertaken in order to examine this relationship in greater detail. First, a 3-year field study of *Haliclona loosanoffi* was made to determine with greater precision the times at which various events in the life history of this sponge occur. Secondly, the germination of gemmules at different temperatures in the laboratory was studied with the hope of gaining some information concerning the regulation of germination. These studies provide evidence for winter diapause in New England populations of *Haliclona loosanoffi*.

The occurrence of gemmules in a related species, *Haliclona oculata*, from Fishers Island Sound is also reported. Although many specimens of this sponge from the northeastern coast of the United States, including specimens from Long Island Sound, have been observed (see de Laubenfels, 1949; Hartman, 1958), there appears to be no previous record of gemmule production. However, Topsent (1888) has briefly described the gemmules of *Haliclona* (*Chalina*) *oculata* on the Channel Coast of France.

Materials and Methods

Field studies

A 3-year study of *Haliclona loosanoffi* was conducted in the Mystic Estuary (Connecticut) in the region north of Mason Island. Observations and collections

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of specimens were made at approximately 2-week intervals during this period. The water temperature was also recorded. From March through December of the last 2 years water samples were taken for salinity determinations. The salinity of filtered samples (Whatman No. 1 filter paper) was measured to the nearest 0.5‰ with a Goldberg refractometer (American Optical).

The specimens were fixed in Bouin's solution in sea water, and each was dissected and examined under a dissecting microscope for the presence of gemmules and embryos (and/or large oocytes). Small samples of the specimens were then embedded in paraffin and sectioned serially at 10 μ. The mounted, deparaffinized sections were stained with hematoxylin and eosin. Examination of such histological sections revealed the stages of development of the gemmules and the presence of small oocytes and spermatic cysts.

Studies of *Haliclona oculata* in Fishers Island Sound (New York) were also made over a 3-year period. Specimens were collected from gravel bottom near the Dumpling Islands in about 30 to 40 feet of water, using 2-bushel oyster dredges. The specimens were preserved on board the boat immediately following their collection. The preservation and examination of the specimens was the same as for *Haliclona loosanoffi*.

Experimental studies

Gemmules of *Haliclona loosanoffi*, encrusting blades of eel grass and algae, were collected during the fall in the Mystic Estuary. Some of the gemmules were stored in the dark at 5° C in covered finger bowls containing sea water; and others were immediately prepared for culturing. The gemmules collected during 1970 were stored in Mystic Estuary sea water, while those collected during 1971 and 1972 were stored in Instant Ocean sea water (Aquarium Systems, Inc.). The Instant Ocean sea water with trace elements was made with glass-distilled water to produce a solution with a salinity of about 24‰. The sea water was changed in the storage vessels at approximately 2-week intervals.

Small pieces of eel grass (*ca* 4 × 3 mm) bearing clusters of gemmules were cultured on sheets of lens paper in covered 4 inch finger bowls containing about 150 ml of Instant Ocean sea water (24‰). Four to 12 such pieces of eel grass were cultured in a single bowl; and the sea water was usually changed at approximately 5-day intervals. In some cases the sea water was changed every other day once germination had begun. The cultures were maintained in the dark at a constant temperature (± 1° C) in B.O.D. incubators.

In most cases when the germination of gemmules under two different temperature regemés was to be compared, blades of eel grass covered by gemmules were cut into pieces across their width; and alternate pieces were put in one of 2 groups. One group of cultures was placed under one set of conditions, and the second group was put under another. In this way the 2 groups were as nearly identical as possible.

The germination of the clusters of gemmules was scored according to 5 arbitrary stages. The characteristics of each of these stages were as follows: stage 1—one or a few small masses of sponge tissue with no oscular tubes; stage 2—more extensive germination, but with less than half of the surface of the culture covered by sponge tissue, no oscular tubes; stage 3—half or more of the surface of the

culture covered by sponge tissue with no oscular tubes; stage 4—less than half of the surface of the culture covered by sponge tissue, but with one or more oscular tubes; stage 5—half or more of the surface of the culture covered by sponge tissue with one or more oscular tubes. The average stage attained by any particular group of cultures is called the germination index (GI). A "P" is used after the germination index to indicate that germination was largely restricted to the edges of the cultures.

Results

Field observations

In order to adequately evaluate experimental results, such as those presented below, detailed information about the occurrence of various events in the life history of *Haliclona loosanoffi* under natural conditions is needed. A population of this sponge was regularly sampled at approximately 2-week intervals for a period extending from March 1969 through February 1972. Other less regular observations were made both before and subsequent to this 3-year period. A preliminary report on this study has already been made (Fell, 1974). Here primary consideration is given to the gemmules; sexual reproduction will be the subject of another publication.

The present study was carried out in the Mystic Estuary, in an area that extends between Mason Island and Pequotsepos Brook. The water in this part of the esutary is shallow, ranging from about 2 to 4 feet in depth. The water temperatures during the winter and summer may differ by more than 25° C. During the months of June through September, the water temperature ranged from 19.0° C to 28.5° C (mean 23.0 ± 2.5° C), while during the months of January and February, the water temperature was generally at or below 0° C (range -2.0° C to 3.0° C, mean -0.5° C). For a major portion of the cold period a layer of ice several inches thick covered the region. From March through May the water temperature rose steadily, and from October through December it sharply declined (see Table I). The salinity generally ranged betwen about 22‰ and 32‰ and was highest during the late summer and early fall (also see Pearcy, 1962).

Table I summarizes some of the observations made on the life history of *Haliclona loosanoffi*. The earliest time of year that active specimens of this sponge have been found in the Mystic Estuary is late May. Such specimens and some ungerminated gemmules were collected on 27 May 1969 and 1 June 1970. In May of 1969 and June of 1969 and 1970 a total of 42 specimens of *Haliclona loosanoffi* were found resting on the bottom. Nearly all of these specimens (39) enclosed empty gemmule capsules, and 9 of them were also associated with yet ungerminated gemmules. It is therefore evident that these specimens developed from gemmules. Pieces of eel grass bearing only ungerminated gemmules or gemmules in the initial stages of germination (Fig. 1) were also observed. The latter were found on 9 June 1969 and 1 June 1970.

Bottom specimens of *Haliclona loosanoffi* were not found until 15 July in 1971 and were not observed at all in 1972. However, such specimens were found on 12 June and 26 June 1973. The observed variation in the occurrence of specimens

derived from gemmules is probably due in part to sampling problems and in part to differences in the production and survival of gemmules from year to year.

Apparently sexual reproduction is initiated soon after the sponges develop from gemmules. The 4 bottom-specimens of *Haliclona loosanoffi* collected in late May 1969 and most (34/38) of the bottom-specimens taken in June 1969 and 1970 possessed oocytes and or embryos or spermatic cysts. Specimens with sexual reproductive elements were found through July.

Both bottom-specimens (many, at least, produced from gemmules) and specimens attached to living eel grass and algae (developed from sexually produced larvae) were found in the study area during most of the summer of some years (1969, 1970 and 1973). Gemmule formation was first observed in late June in

TABLE I

The occurrence of gemmules and active specimens of Haliclona loosanoffi *in the Mystic Estuary*

Month	Water temp. °C*	Active sponges	Gemmules			
			Developing	Formed	Exposed	Germinating
Jan.	−0.5	−	−	+	+	−
Feb.	0	−	−	+	+	−
March	5.5	−	−	+	+	−
April	10	−	−	+	+	−
May	16	+	−	+	+	+**
June	22	+	+	+	+	+
July	24	+	+	+	−	?
Aug.	24	+	+	+	±	−
Sept.	21	+	+	+	±	−
Oct.	16	±	+	+	+	−
Nov.	7.5	±	−	+	+	−
Dec.	2.5	−	−	+	+	−

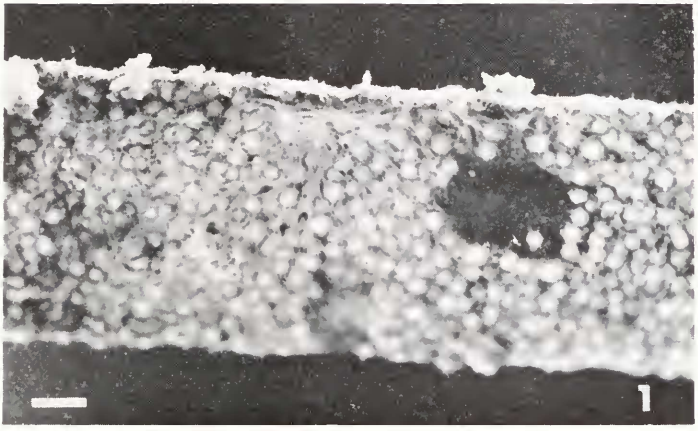
* Mean surface water temperature at low tide.

** Not actually observed; but since some sponges have been found in May, some germination must occur during this month.

some of the bottom-specimens and in late July in the sexually produced specimens attached to eel grass. Thus this process may begin within about one month after the germination of the gemmules produced during the preceding year. The production of gemmules was found to continue into October, the substrates of the sponges being progressively covered with gemmules as the specimens grow.

Regression began in some specimens of *Haliclona loosanoffi* by late August and early September; and some gemmules were exposed at this time. By early October there were many pieces of eel grass and algae bearing exposed gemmules. Many of these gemmules were completely exposed, while others were partially covered by a latticework of bare parental skeleton. Living specimens of *Haliclona loosanoffi* were associated with some of these gemmules, but not with many others. Many of the specimens showed evidence of degeneration. In some cases the sponge tissue was retracted slightly away from the basal gemmules, and in other cases it was separated from them by a broad zone of vacated skeleton (Fig. 2).

Furthermore, many of the specimens found at this time of year were a purplish color instead of the usual rosy beige or tan. The latest that "active" specimens of *Haliclona loosanoffi* were observed in the Mystic Estuary was mid-November. A few small lavender-colored specimens were found on 14 November 1970, but no active specimens were observed during this month in other years.



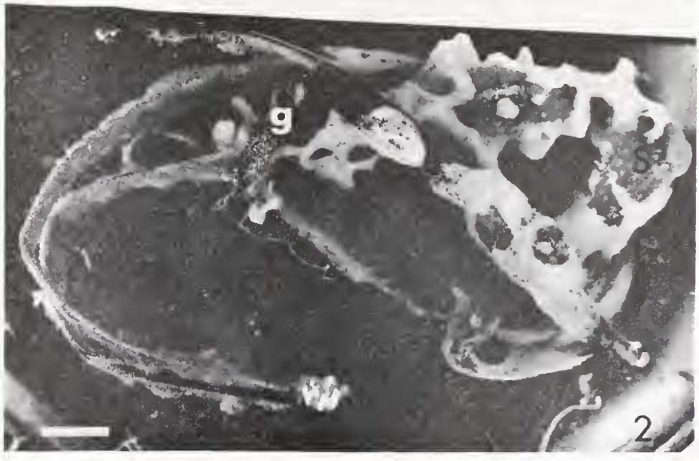


FIGURE 1. Living gemmules of *Haliclona loosanoffi* in an early stage of germination, collected on 9 June 1969 (scale bar = 1 mm).

FIGURE 2. Preserved specimen of *Haliclona loosanoffi* collected on 11 October 1969. Note the exposed gemmules (g) and vacated skeleton (s) (scale bar = 10 mm).

Gemmules were the only form in which *Haliclona loosanoffi* was found from November through late May. These gemmules encrusted pieces of dead eel grass and algae; and in some cases they were covered by the tissue of another sponge, *Halichondria bowerbanki,* which is sometimes abundant during the winter. The structure of the gemmules of *Haliclona loosanoffi* has been described by Hartman (1958) and Simpson and Fell (1974).

Germination of fall gemmules at 20° C

Gemmules collected on 4 October 1971 were used to prepare 50 cultures which were divided into two equal groups (A-1 and A-2). Both groups were initially placed at 20° C. After about one week most of the cultures showed germination of the gemmules along their edges, and many of the resulting sponges possessed

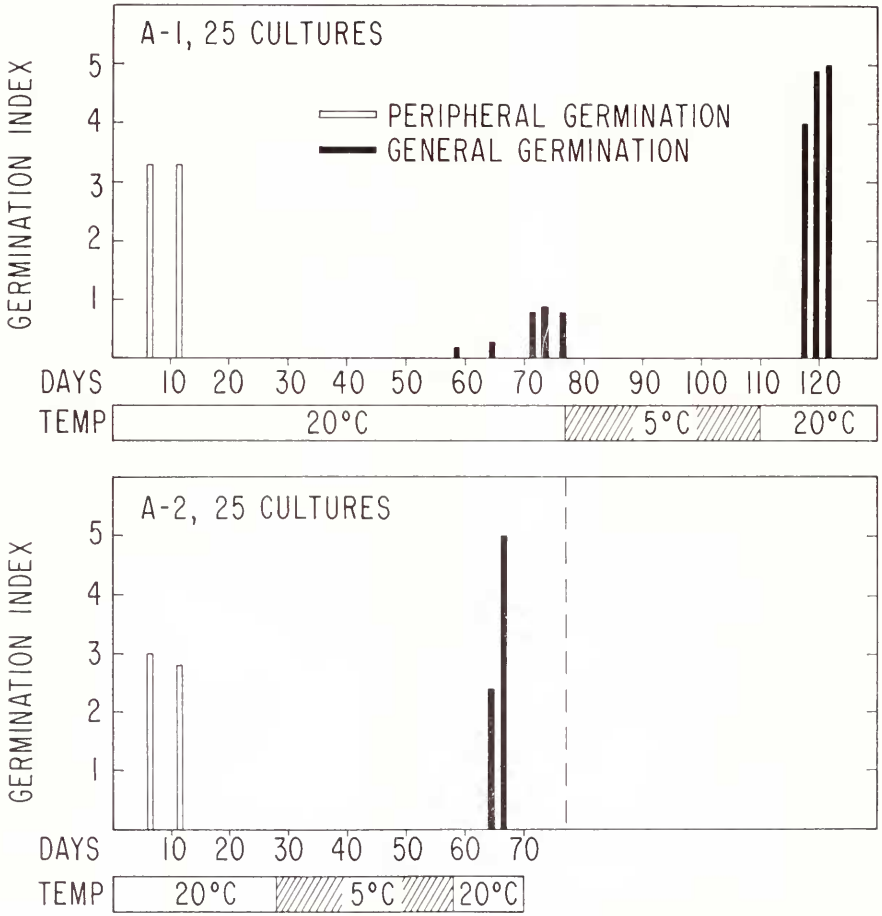


FIGURE 3. Germination of gemmules, collected on 4 October 1971, at 20° C before and after a 4-week period at 5° C.

oscular tubes (stage 4P). The germination index was 3.3P (see Fig. 3). After about 20 days many of the sponges showed signs of degeneration. The oscular tubes disappeared, and the sponge tissue began to gradually waste away. At this time all of the cultures still contained many ungerminated gemmules.

The occurrence of germination along the edges of the cultures and especially along the cut edges was very striking (see Fig. 4). On the 9th day of culture 82% of the cut edges bore sponges compared with only 18% of the uncut edges

and 22% of the upper surfaces. This pattern of germination suggests that physical damage to some of the cells of the gemmules and/or to the gemmule capsules may in some way stimulate the gemmules to germinate. This suggestion is strengthened by the fact that cultures of gemmules, which have no cut edges, do not exhibit such preferential germination at their periphery (see below).

On the 28th day one group of cultures (A-2) was placed at 5° C for 30 days. At the end of the cold treatment it was returned to 20° C. Seven days after the cultures were placed at the higher temperature, all of them had begun to germinate; and by the 9th day all of them had reached stage 5 (see Figures 3 and 5). Germination was therefore essentially complete on the 67th day of total culture. In the other group of cultures (A-1), which was kept at 20° C, the first indication of later germination was on day 59. Eventually 5 of the 25 cultures showed some germination (GI = 0.9), after which the sponge tissue began to regress. On day 77, which was 10 days after all of the A-2 cultures had reached stage 5, the A-1 cultures were placed at 5° C for 33 days. Then they were returned to 20° C. By the 12th day at 20° C (the 122nd day of total culture), all 25 cultures were at stage 5 (see Fig. 3).

In another experiment 17 cultures were prepared using large, uncut pieces of eel grass covered by gemmules (average size, *ca* 34 × 4 mm). The masses of gemmules, which were collected on 28 October 1971, were divided into 2 groups. One group of 9 cultures (B-1) was placed initially at 20° C; and the other group (B-2), which consisted of 8 cultures, was cultured at 5° C for one month before being placed at 20° C.

The B-1 cultures did not experience an early, peripheral germination. By day 29 only 2 cultures showed any signs of germination. Both of these cultures were at stage 1 (GI = 0.2). After 39 days the germination index was only 1.1; on day 46 it was 3.1; and on day 55 it was 3.7. In all of the cultures there were still many ungerminated gemmules. On the 55th day the cultures were placed at 5° C for 41 days. At the end of the cold treatment the cultures were returned to 20° C. By the 11th day at 20° C (the 107th day of total culture) all of the cultures had reached stage 5.

The B-2 cultures were transferred from 5° C to 20° C after 31 days of culture. By the 12th day at the higher temperature (the 43rd day of total culture) the germination index was 4.4. Five of the 8 cultures produced large sponges, but all of them still possessed many ungerminated gemmules.

The results of these experiments suggest that gemmules collected during the early fall do not readily germinate at 20° C and that low temperature enhances germination of the gemmules. Additional support for these conclusions is given by the experiment described in the next section.

Germination of fall gemmules at 20° C following a short period at 5° C

Four groups of cultures were set up using gemmules collected on 4 October 1971. These groups consisted of gemmules which had been stored at 5° C for different lengths of time (1, 2, 5 and 9 weeks) before being cultured at 20° C. All of the cultures derived from the same mass of gemmules were placed in the same finger bowl so that differences between different masses of gemmules (at least 3 per group) could be detected. Table II summarizes the results of this experiment.

Twenty eight cultures were set up using gemmules which had been kept at 5° C for one week. These were divided into 2 groups: group BA-1 containing 18 cultures and group BA-2 containing 10 cultures. When the cultures were placed at 20° C, they behaved like cultures which had received no cold treatment. After about one week there was extensive germination along the edges of the cultures (GI = 3.6P), but all of the cultures still contained many ungerminated gemmules. Again most of the germination occurred along the cut edges of the cultures. On the 7th day 87% of the cut edges were covered by small sponges, while only 29% of the uncut edges and 25% of the upper surfaces of the cultures bore sponges. Over a period of several weeks, the sponges in these cultures gradually wasted away.

On the 28th day the BA-1 cultures were placed at 5° C for 28 days and then returned to 20° C. By the 11th day at 20° C (the 67th day of total culture) most of the cultures exhibited extensive germination, and the germination index

TABLE II

The effect of a period(s) at 5° C on the subsequent germination of gemmules at 20° C (all gemmules collected 4 October 1971); germination index: (A) initial germination at 20° C, (B) subsequent germination by end of temperature régime, (C) germination after a later 4-week cold treatment

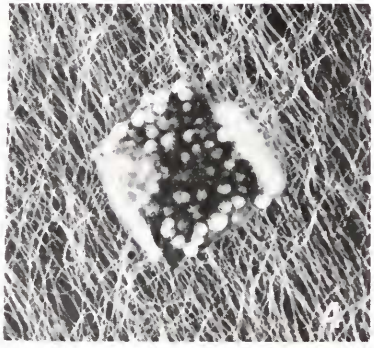
Exp.	No. cultures	Temperature régime*	Germination index		
			A	B	C
A-2	25	*4–4*–1	(3.3P)	5.0	—
A-1	25	10		0.9	5.0
BA-1	18	*1–4–4*–2	(3.6P)	4.7	—
BA-2	10	*1*–10		0.8	5.0
BB-1a	10	*2*–1	5.0	—	—
BB-1b	10	*2–4–3*–2	4.2 (3.5)	5.0	—
BB-2	12	*2*–9		1.3	5.0
BC	33	*5*–2	4.0	—	—
BD	30	*9*–2	4.9	—	—

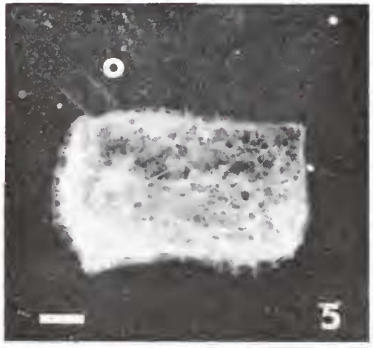
* Number of weeks at 20° C in regular type; number of weeks at 5° C in italics.

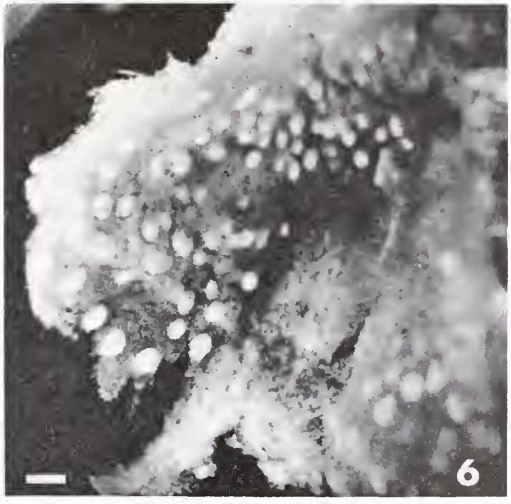
was 4.7. At this time the germination index of the BA-2 cultures, which had been kept continuously at 20° C, was only 0.8. On day 71 the BA-2 cultures were placed at 5° C for 31 days. At the end of the cold treatment the cultures were returned to 20° C, and by the 7th day at the higher temperature all of them had reached stage 5 (see Table II).

A group of 8 cultures (BA-3) was prepared at the same time as the other two, using gemmules kept at 5° C for one week. The capsules of many of the gemmules in this group were pierced with a sharp iridectomy knife. After about a week at 20° C, 7 of the cultures showed extensive germination; and the germination index was 4.75. Admittedly the number of cultures is small, but the results lend further support to the suggestion that damage to the gemmules may cause early germination. Although many of the gemmules in this group of cultures germinated within 2 weeks, many others did not germinate until after they had received a 31-day cold treatment.

Thirty two cultures were prepared from gemmules which had been stored at 5° C for 2 weeks. These were divided into one group of 20 cultures (BB-1) and another group of 12 cultures (BB-2). Both groups of cultures were initially placed at 20° C. By the 8th day 14 cultures were at stage 5, and the germination index was 4.2. Ten of the stage-5 cultures (part of group BB-1) were from a







FIGURES 4 and 5. Cultures of gemmules collected on 4 October 1971: 4. 8-day culture at 20° C showing germination along the cut edges of the culture; 5. stage-5 culture on the 13th day at 20° C following a long period at 5° C. Note the oscular tube (o) (scale bar = 1 mm).

FIGURE 6. Surface of a specimen of *Haliclona oculata* that was formerly in contact with the substrate. Note the gemmules. Specimen was collected on 6 June 1972 (scale bar = 2 mm).

single blade of eel grass. Excluding these cultures, the germination index was 3.5 or about the same as that for the cultures kept at 5° C for one week prior to being placed at 20° C. However, in this set of cultures there was little peripheral germination; only 10 of the 32 cultures had sponge tissue restricted to their periphery.

The 10 stage-5 cultures of group BB-1, which were from the same mass of gemmules, were discarded since most, if not all, of the gemmules had apparently germinated. After the remaining BB-1 cultures had been at 20° C for 30 days, they were placed at 5° C for 18 days. At the end of the cold treatment the cultures were transferred back to 20° C. By the 13th day at the higher temperature (the 61st day of total culture) all of the cultures were at stage 5. On the other hand, the BB-2 cultures, which had not had a second exposure to low temperature, had a germination index of only 1.3. On the 64th day of culture the BB-2 cultures were placed at 5° C for 31 days, and then they were transferred back to 20° C. After 9 days at 20° C all of these cultures had reached stage 5 (see Table II).

Gemmules, which had been stored at 5° C for 5 weeks, were used to set up 33 cultures. After about 2 weeks at 20° C the cumulative germination index was 4. Although in this case the germination index was somewhat low, in other experiments a 4 to 6 week period at 5° C was sufficient to produce nearly complete germination. This was true in experiments A, BA and BB (see Table II).

TABLE III

The germination of cold-treated gemmules at various temperatures (all gemmules collected in October)

Culture temp. °C	No. cultures	Weeks stored at 5° C	Days to max. germination	Germination index
10	34	43	—	0
15	42	30 & 39	avg. 22	5.0
20	180	4–9	avg. 11	4.73
20	146	30–41	avg. 10	4.96
25	35	41	12	5.0
30	69	31 & 35	avg. 10	3.96
34	18	38	—	0

Thirty cultures were set up using gemmules which had been stored at 5° C for 9 weeks. By the 11th day at 20° C nearly all of the cultures were at stage 5, and the germination index was 4.9. Although most of the cultures produced large sponges, there were still a number of ungerminated gemmules in many of the cultures.

Germination of fall gemmules at various temperatures after a long period at 5° C

Gemmules, which had been collected in October and stored at 5° C for from 30 to 43 weeks, were tested for their capacity to germinate at 10, 15, 20, 25, 30 and 34° C. In most experiments the germination of gemmules at 20° C was compared with that at some other temperature. The results of these experiments are summarized in Table III.

Thirty four cultures, which were kept at 10° C for 30 days, showed no evidence of germination. That the gemmules were healthy and capable of germination under favorable conditions was demonstrated by their response when they were transferred to 20° C. By the 9th day at the higher temperature all of the cultures had reached stage 5.

A total of 42 cultures were kept at 15° C. In one experiment involving 30 cultures, the first evidence of germination was on the 15th day and it was not until the 27th day that all of the cultures had reached stage 5. In another experiment, 12 cultures kept at 15° C all reached stage 5 by day 17. While the time required for germination was less than in the first experiment, it was substantially greater than that for 12 control cultures kept at 20° C. All of the latter cultures reached stage 5 on the 8th day of culture.

As has already been indicated, gemmules, which have had at least a 4-week cold treatment, germinate within about one to 2 weeks at 20° C. In a number of experiments involving over 300 cultures, the maximal germination index was usually achieved within 8 to 12 days, and 50% or more of the cultures showed evidence of germination within 5 to 7 days (see Table III).

Thirty five cultures kept at 25° C behaved essentially like cultures kept at 20° C, except that germination was accelerated by about 24 to 48 hrs. On the 5th day most of the cultures placed at 25° C were in early stages of germination, and one day later 28 of them were at stage 5. By comparison, only 4 of 35 control cultures kept at 20° C had begun to germinate by the 5th day, and none of them were at stage 5 on day 6. However, on the 7th and 8th days of culture the number of cultures to have reached stage 5 was 18 and 28 respectively.

A total of 69 cultures were placed at 30° C. Many of these cultures reached stage 5, but their development was inferior to that of gemmules kept at 20 or 25° C. In one experiment, involving 38 cultures, about half of the cultures reached stage 5; and the germination index was 3.7. In a second experiment, using 31 cultures, the germination index was 4.3. Cotnrol cultures (30) kept at 20° C were delayed in their germination by about 24 hrs compared to the cultures kept at 30° C, but all of them reached stage 5 by the 8th day of culture. Not only did more cultures reach stage 5 at 20° C, but the cultures appeared to be more robust. Twelve cultures of the second experiment, which did not reach stage 5 (GI = 3.2), were subsequently transferred to 20° C. After 9 days at the lower temperature the germination index of this group of cultures was 4.9.

Twenty nine cultures were placed at a variable high temperature (31.0 to 34.5° C). By the 8th day of culture only 10 cultures (all from one group of gemmules) showed any evidence of germination. On the other hand, 29 control cultures kept at 20° C had all reached stage 5 by this time. Already by the 7th day of culture sponge tissue, which resulted from the limited germination at high temperature, was beginning to regress. On the 8th day the remaining sponge tissue was removed from the gemmules with a sable brush, and the cultures were placed at 20° C. By the 13th day at 20° C (the 21st day of total culture) 27/29 cultures had attained stage 5 (GI = 4.8). This experiment suggests that the germination of the gemmules of *Haliclona loosanoffi* may be reversibly inhibited by high, as well as by low, temperature.

Finally, 18 cultures were kept at 34° C. These cultures showed no evidence of germination during 20 days at this temperature; and when they were subsequently transferred to 20° C, there was no germination during a 16-day period. By comparison most of the 18 control cultures placed at 20° C were in early stages of germination on the 6th day and had reached stage 5 by day 12 (GI = 4.9). This result suggests that a prolonged exposure of the gemmules to 34° C is lethal.

Additional observations

In a number of cultures it was noted that little or no germination occurred among the gemmules situated on the lower surface of the eel grass. However, when the cultures were recultured in an inverted position, there was frequently extensive germination among these gemmules. This observation suggests that conditions are less favorable for germination on the undersurfaces of the cultures where the gemmules are in contact with the substrate. This may be due to a restriction of respiratory gas exchange and/or an accumulation of certain metabolic byproducts.

It appears that the gemmules of *Haliclona loosanoffi* can be stored at 5° C under the conditions used in this study for only about one year. In all, 112 cultures were prepared from gemmules collected on 8 October 1970 and stored at 5° C for from 7 to 10 months. These cultures, which were kept at from 15 to 30° C, had a germination index of 4.7. The only cultures, which did not reach stage 5, were some of those kept at 30° C. On the other hand, 90 cultures derived from gemmules collected on the same date and stored at 5° C for from 16.5 to 19.5 months had a total germination index of only 0.1 when cultured at 20 or 25° C.

Finally, many of the gemmules, kept at 10 to 20° C for long periods of time without germination, became covered by a thin, dark brown layer of what appeared to be algal growth. However, this layer did not seem to significantly hinder the subsequent germination of the gemmules.

The gemmules of Haliclona oculata

More than 300 specimens of *Haliclona oculata* were dredged from Fishers Island Sound in the vicinity of the Dumpling Islands. Collections were made at irregular intervals over a period extending from May 1971 to February 1974 and during every month except September. In all of the 18 collections, many of the sponges (40 to 100%, avg. 76%) possessed gemmules. These were situated at the base of the stalk, either in contact with the substrate or only a few millimeters above it (Fig. 6). From March through June many of the specimens possessed oocytes, embroys, and/or larvae in progressively advanced stages of development. In some cases these occurred throughout the endosome from the fibrous stalk to the tip of the sponge. Some of the specimens, which lacked oocytes and embryos, were found to contain large numbers of spermatic cysts.

In many of the collections, a few of the specimens showed evidence of basal degeneration. In some cases the stalk was devoid of living tissue, but most of the rest of the specimen appeared to be healthy. In other cases the dead stalk supported only a few small masses of sponge. Dead stalks alone were also found. What appeared to be living gemmules were observed at the base of some of the dead stalks.

The number of gemmules produced by any specimen was relatively small. An exact enumeration of the gemmules was difficult, because they were frequently embedded in a dense network of spongin (collagen) fibers. However, it appeared that some of the specimens possessed fewer than 10 gemmules, while others contained as many as 70 or more. This is in general agreement with the observations of Topsent (1888); however, the estimate given by him that 30 is the

maximal number of gemmules produced by a single specimen of this sponge is low. Although one specimen 57 mm in height was found to possess gemmules, it appears that these structures are generally restricted to larger specimens (> *ca* 80 mm in height). The gemmules were generally from about 600 μ to more than 1000 μ in their greatest diameter.

An attempt to bring about the germination of the gemmules of this sponge was unsuccessful. Fifteen groups of gemmules, collected on 4 May 1973, were placed in finger bowls in an aquarium (Dayno Aqua Lab) containing 20 gallons of freshly collected sea water (30‰) that was constantly circulated. One to 3 groups of gemmules were placed in each finger bowl. Four of the groups of gemmules were collected with dead stalks, and the rest were obtained from living specimens. The water in the aquarium was maintained at 8° C during the first 48 hours, after which it was allowed to come up to room temperature (*ca* 23° C). During 28 days of culture there was no evidence of germination, although the gemmules of *Haliclona loosanoffi* readily germinate within a few days under similar conditions (Olmstead and Fell, unpublished).

Discussion

Haliclona loosanoffi and *Haliclona oculata* are among at least five species of *Haliclona* that are known to produce gemmules. The other species are *Haliclona* (*Chalina*) *gracilenta* (Topsent, 1888); *Haliclona ecbasis* (Fell, 1970); and *Haliclona permollis* (David Elvin, Oregon State, personal communication). However, not all haliclonids form gemmules. For example, in Long Island Sound *Haliclona canaliculata* overwinters in a simplified form lacking flagellated chambers, but does not produce gemmules (Hartman, 1958). Other gemmuliferous marine sponges include *Suberites domuncula* (Herlant-Meewis, 1948), *Suberites ficus* (Topsent, 1888; Hartman, 1958), *Prosuberites microsclerus* (Wells *et al*, 1964), *Laxosuberites lacustris* (Annandale, 1915), *Cliona vastifica* (Topsent, 1888; Annandale, 1915), and *Cliona truitti* (Wells *et al*, 1964).

A number of other sponges, including *Haliclona heterofibrosa* (Bergquist, Sinclair and Hogg, 1970), apparently produce gemmule-like masses which develop into free-swimming parenchymula larvae. Such "gemmules" usually are not in contact with the substrate of the sponge, and they do not possess a thick enveloping capsule. Although this form of asexual reproduction was first described by Wilson in 1891 and 1894, it remains poorly understood (see Fell, 1974).

The gemmules of *Haliclona loosanaffi* are frequently present throughout most of the year in the Mystic Estuary. They are produced beginning in June or July and do not germinate until the following May or June. During the late summer and early fall the parent sponges degenerate, leaving the gemmules attached to the substrate. The gemmules are the only form in which this sponge exists during the colder months of the year, and consequently at this location they are an obligatory part of the life history. Evidently the gemmules of this sponge are special structures which permit it to survive low temperature and other adverse conditions (also see Hartman, 1958).

In this study it has been shown that the gemmules of *Haliclona loosanoffi* may be stored at 5° C for up to 10 months without showing any signs of germination. However, such gemmules readily germinate when they are subsequently

cultured at 20° C. Gemmules collected in early October have germinated in the laboratory as early as December and as late as September. Germination also occurs at 15° C but not at 10° C. Well developed sponges usually develop in about one to 2 weeks at 20° C or in approximately 2 to 4 weeks at 15° C. The results of these laboratory studies are in good agreement with field studies which suggest that germination normally occurs as the water temperature is rising from about 15 to 25° C. It therefore appears that germination of the gemmules is prevented during the winter by low temperature. A similar situation has been found to exist for the gemmules of several fresh-water sponges (Rasmont, 1954, 1962 and 1963; Strekal and McDiffett, 1974). However, the gemmules of *Spongilla lacustris* and *Trochospongilla (Tubella) pennsylvanica* (in Northern New England) normally germinate when the water temperature is still only 4 to 5° C (Simpson and Gilbert, 1973).

The regulation of gemmule germination in *Haliclona loosanoffi* is not as simple as it first appears. The gemmules begin to form early in the summer, and the completed gemmules become exposed on the substrate at the end of the summer when the water temperature may be close to 20° C or about the same as that occurring in the spring when the gemmules normally germinate. Some mechanism(s) must therefore be operating to prevent the gemmules from germinating prior to the onset of winter conditions.

Gemmules of *Haliclona loosanoffi*, collected in the early fall and cultured at 20° C without a period of storage at 5° C, do not germinate as readily as cold-treated gemmules. In a number of experiments, involving gemmules with either no or only a very short cold treatment, few of the gemmules germinated during approximately 2 months of culture at 20° C. On the other hand, cultures of gemmules, which had been kept at 5° C for about 4 weeks before being placed at 20° C, underwent extensive germination within a period of 2 weeks. These experiments suggest that some process(es) in the germination of the gemmules requires a relatively long period of time and that this process is accelerated by, but is not totally dependent upon low temperature. Such a condition is known as diapause (Agrell, 1951; Rasmont, 1954).

The diapause experienced by the gemmules of *Haliclona loosanoffi* appears to be very similar to that occurring in the gemmules of the fresh-water sponge, *Ephydatia mülleri*. Gemmules of the latter species, collected in July and maintained for nearly 4 months at 3.5° C, showed 90% germination after 8 days at 20° C, while gemmules, kept at 14 to 16° C for the same period, exhibited only 1% germination after 8 days and 11.5% germination after 20 days at 20° C (Rasmont, 1954). The timing of certain events in the life histories of these sponges is also similar. Near Brussels, Belgium *Ephydatia mülleri* reproduces sexually in May, produces gemmules primarily during June and July, and regresses, except for gemmules, by early fall (Rasmont, 1962). The gemmules of *Spongilla fragilis* and the brown gemmules of *Spongilla lacustris* also undergo diapause, but in these cases the diapause appears to be less deep (Rasmont, 1954 and 1955).

The exposure of the gemmules of *Haliclona loosanoffi* to 5° C for a period of one week has little effect on subsequent germination compared to that of unchilled gemmules. However, a cold treatment of only 2 weeks appears to result in a definite but variable enhancement of germination; and exposure to 5° C for 4 or more weeks usually leads to the germination of most of the gemmules when they

are then cultured at 20° C. Similarily, Rasmont (1954) found that 3-day exposure to 3.5° C had no effect on the subsequent germination of the gemmules of *Ephydatia müller*i, but that treatments of 12, 20 and 30 days resulted in progressively higher percentages of germination, 45%, 75% and 92% of the gemmules respectively.

The optimal temperature for breaking diapause in the gemmules of *Haliclona loosanoffi* has not yet been determined. However, it has been shown that 3° C and 8° C are equally effective in bringing about germination of the gemmules of *Ephydatia müller*i (Rasmont, 1955). A temperature of 12° C was somewhat less effective than the lower temperatures but was substantially more effective than 18° C. Gemmules collected on 1 September and exposed to 3 or 8° C for one month subsequently showed 90% germination when they were cultured at 20° C. By comparison 60% of the gemmules exposed to 12° C and only 25% of those exposed to 18° C germinated when tested under the same conditions. However, it was found that after 2 to 3 months at 18° C, most of the gemmules were capable of germination. A similiar situation was shown to exist for the gemmules of *Spongilla fragilis*, but for this species 8° C was somewhat more effective than either 3 or 12° C in breaking diapause (Rasmont, 1955). If the gemmules of *Haliclona loosanoffi* are physiologically similar to those of these fresh-water sponges, 5° C should be close to the optimal temperature leading to the maturation of the gemmules.

When and how the diapause is initiated are unknown. One would like to know whether it is initiated when the gemmules are formed or at some later time. Also nothing is presently known concerning the nature of the diapause itself. However, one observation on the germination of gemmules, which had received no cold treatment or only a very short one, is of interest in this connection. Frequently many of the gemmules situated along the cut edges of the cultures germinated within a few days, while most of the rest of the gemmules did not germinate even after a long period of time. This suggests that injury to the gemmule capsule and/or to the cells of the gemmule may stimulate germination. Perhaps the gemmule capsule, like the coats of certain seeds, is important in maintaining dormancy either by restricting respiration or by retarding the loss and/or inactivation of germination inhibitors (Roberts, 1969).

Ephydatia fluviatilis and *Spongilla lacustris* produce an inhibitor of gemmule germination (so-called gemmulostasin), but *Ephydatia müller*i does not (Rasmont, 1965; Rosenfeld, 1970). The first 2 species form gemmules late in the year and do not degenerate until the onset of winter conditions (Rasmont, 1962). The inhibitor produced by the parental tissue inhibits the germination of the gemmules until this time; and the gemmules of *Ephydatia fluviatilis* and the green gemmules of *Spongilla lacustris*, unlike those of *Ephydatia müller*i, do not undergo diapause (Rasmont, 1954 and 1962). The inhibitors of *Ephydatia fluviatilis* and *Spongilla lacustris* are not species specific and inhibit the germination of the gemmules of *Ephydatia müller*i. That of *Ephydatia fluviatilis* acts on an early phase of germination; and once this phase is past, the inhibitor has no effect on the later development of the new sponge. As would be expected, the effect of the inhibitor is reversible (Rosenfeld, 1970). It is of interest that if the capsule of the gemmules is pierced, germination is not inhibited by gemmulostasin although it is slowed compared to that of untreated controls (Rosenfeld,

1971). From the preceding discussion one would predict that *Haliclona loosanoffi* does not produce such an inhibitor of gemmule germination. Certainly if it does, the inhibitor could play only a minor regulatory role.

At Hatteras Harbor, North Carolina some specimens of *Haliclona loosanoffi* degenerate during the winter, but many others are present throughout this period when the water temperature falls to about 5° C (Wells *et al.*, 1964). Although the winter at Hatteras Harbor is apparently less severe than at Mystic, there presently seems to be no obvious explanation for the survival of *Haliclona loosanoffi* at the former location but not at the latter. In the Mystic Estuary the sponges degenerate in the late summer and early fall when the water is still warm. The gemmules exposed during the winter at Hatteras Harbor germinate in April when the water temperature reaches approximately 16° C, a situation similar to that in New England (Wells *et al.*, 1964).

On the other hand, at Hatteras Harbor *Haliclona loosanoffi* is absent, except for gemmules, during the middle of the summer when the water temperature is about 30° C. The summer gemmules, which germinate is September, may be of 2 types: those produced during the fall and winter and exposed to winter temperatures while enclosed by parental sponge tissue and those produced during the late spring and early summer (Wells *et al.*, 1964). It would be of interest to know whether these 2 classes of gemmules differ in their capacity to germinate. One class of gemmules was chilled and the other was not. However, the chilling of gemmules within the parental sponge may not have the same effect as that of chilling exposed gemmules.

The gemmules of *Haliclona oculata* appear to play a different biological role from that played by the gemmules of *Haliclona loosanoffi*. Specimens of the former sponge evidently remain active and gemmules apparently are present throughout the year. The gemmules may repopulate the substrate when specimens degenerate or when the sponges are torn loose during storms. Herlant-Meewis (1948) believes that the gemmules of *Suberites domuncula* play a similar role.

Although the present report is primarly concerned with the gemmules of *Haliclona loosanoffi* and *Haliclona oculata*, some information concerning sexual reproduction is given. In the Mystic Estuary specimens of *Haliclona loosanoffi* with reproductive elements are found in May, June and July (also see Fell, 1974). Larvae may be released as early as the middle of June, and the peak of larval settlement appears to occur in July (Fell, in preparation). The timing of reproduction is very different from that reported for this species at Milford, Connecticut (Hartman, 1958). At the latter location oocytes and embryos are present in specimens collected during late August and early September and the peak of larval settlement occurs during late September and early October. It is somewhat surprising that the reproductive periods of this sponge at 2 localities as near to each other as Mystic and Milford should be so different. At Hatteras Harbor, North Carolina *Haliclona loosanoffi* has 2 periods of larval settlement, one during June and July and the other in October and November (Wells *et al.*, 1964).

At Mystic, Connecticut and Hatteras Harbor, North Carolina (Wells *et al.*, 1964) sexual reproduction is initiated very soon after sponges develop from gemmule germination. A similar situation has been found to exist for the fresh-

water sponges, *Spongilla lacustris* and *Trochospongilla (Tubella) pennsylvanica* (Simpson and Gilbert, 1973). Furthermore, in *Spongilla lacustris* oocytes begin to develop within one week after refrigerator-stored gemmules are inplanted back into the natural habitat of the sponge, even when this is done well after the normal period of sexual reproduction (Gilbert, 1974). These facts suggest that there may be a regulatory connection between sexual reproduction and gemmule germination. However, gemmulation does not appear to be an obligatory prerequisite to sexual reproduction in these species. Larva-derived specimens of *Spongilla lacustris* (Simpson and Gilbert, 1974) and *Haliclona loosanoffi* (Fell, in preparation) may exhibit low levels of gamete production.

It is reported here that the reproductive period of *Haliclona oculata* in Fishers Island Sound extends from March through June. The only other reference to the reproductive period of this sponge is that of Hartman (1958). He reported the occurrence of reproductive specimens in Block Island Sound during July.

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SUMMARY

1. The gemmules of *Haliclona loosanoffi* are present throughout the year in the Mystic Estuary, but active specimens of this sponge are found only during the period extending from late May to late October or early November. Germination of the gemmules occurs primarily during May and June, and new gemmules are produced from late June through early October. The gemmules of this species evidently are a means for surviving adverse environmental conditions.

2. Both gemmules and active specimens of *Haliclona oculata* are found throughout the year in Fishers Island Sound. The gemmules of this species may repopulate the substrate when the parent sponges degenerate or are ripped loose during storms.

3. Gemmules of *Haliclona loosanoffi* collected in the fall do not germinate readily when they are cultured at 20° C. There is frequently only limited germination after 1 to 2 months at this temperature. However, if the gemmules are first put at 5° C for 4 or more weeks, they usually germinate within a few days after being placed at 20° C. Thus low temperature appears to enhance germination of these gemmules which undergo diapause.

4. Although low temperature appears to accelerate maturation of the gemmules of *Haliclona loosanoffi*, it also inhibits actual germination. Gemmules do not germinate at either 5 or 10° C. However, germination occurs within 2 to 4 weeks at 15° C and within 1 to 2 weeks at 20° C. At 25° C germination appears to be accelerated by approximately 24 to 48 hours compared to that occurring at 20° C.

5. The gemmules of *Haliclona loosanoffi* may be stored in the dark at 5° C for at least 10 months without any detectable reduction in their capacity to germi-

nate at higher temperatures. However, such gemmules can not be stored indefinately. Gemmules kept at 5° C for from 16.5 to 19.5 months did not germinate when they were subsequently placed at either 20 or 25° C.

6. Gemmules of *Haliclona loosanoffi* germinate at 30° C, but this temperature appears to be less favorable than 20 or 25° C. Slightly higher temperatures inhibit germination, and continuous exposure to 34° C apparently is lethal.

7. The reproductive period of *Haliclona loosanoffi* in the Mystic Estuary extends from late May through July and that of *Haliclona oculata* in Fishers Island Sound extends from March through June.

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